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Vasopressin and Oxytocin Gene Expression in the Human Hypothalamus

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ABSTRACT

We studied the distribution of messenger ribonucleic acids coding for vasopressin and oxytocin in the human hypothalamus by means of hybridization histochemistry. Numerous large and medium-sized neurons contain vasopressin messenger ribonucleic acid in the paraventricular nucleus, supraoptic nucleus, and accessory magnocellular nucleus. Small, lightly labeled vasopressin neurons also were detected in the suprachiasmatic nucleus. In addition, a relatively sparse band of mostly ovoid, medium-sized vasopressin neurons mingle with unlabeled neurons of the lateral hypothalamic area; these cells extend dorsoventrally from the region ventral to the stria terminalis to the ventrolateral hypothalamus, sometimes transgressing the boundaries of nearby nuclei. We did not detect vasopressin gene expression in neurons of the bed nucleus of the stria terminalis proper, although some of the dorsal-most labeled neurons of the lateral hypothalamus extend into the region of the caudal bed nucleus. Some lateral hypothalamic neurons also encroach upon other extrahypothalamic structures, such as the zona incerta. The nucleus basalis of Meynert complex was, with only rare exceptions, devoid of cells containing vasopressin messenger ribonucleic acid.

Oxytocin messenger ribonucleic acid is found in the supraoptic nucleus, paraventricular nucleus, accessory magnocellular nucleus and, less frequently, in neurons of the lateral hypothalamus. In the hypothalamic magnocellular nuclei, oxytocin neurons are somewhat smaller than vasopressin neurons. Vasopressin cells outnumber oxytocin cells in the supraoptic nucleus, but their numbers are comparable in the paraventricular nucleus. As with vasopressin neurons, lateral hypothalamic oxytocin cells loosely span several diencephalic nuclei and encroach occasionally upon adjacent regions. These results confirm that the organization of vasopressin and oxytocin neurons in the human hypothalamus is largely comparable to that in nonhuman species and demonstrate the utility of hybridization histochemistry for elucidating the chemoarchitecture of the human brain.

Key words: basal forebrain, paraventricular nucleus, in situ hybridization histochemistry, suprachiasmatic nucleus, supraoptic nucleus

Vasopressin and oxytocin are well-characterized nonapeptides present in magnocellular neurons of the hypothalamic supraoptic nucleus (SON) and paraventricular nucleus (PVN; for review see Young, '92). Both of these peptides are synthesized in the hypothalamus as preprohormones and are processed during axonal transport for storage in, and release from, the neurohypophysis (Brownstein, '80). Vasopressin promotes the reabsorption of water by the kidney and plays an important role in cardiovascular regulation in some species (Berecek and Swords, '90). Oxytocin is involved in parturition and lactation. Vasopressin and oxytocin also have been implicated in the stress response (Antoni et al., '88; Samson and Mogg, '90), sexual arousal and ejaculation (Murphy et al., '87), and cognitive processes (Bohus et al., '78; Ferrier et al., '80; Beckwith et al., '82; Kennett et al., '82). Vasopressin neurons are affected in

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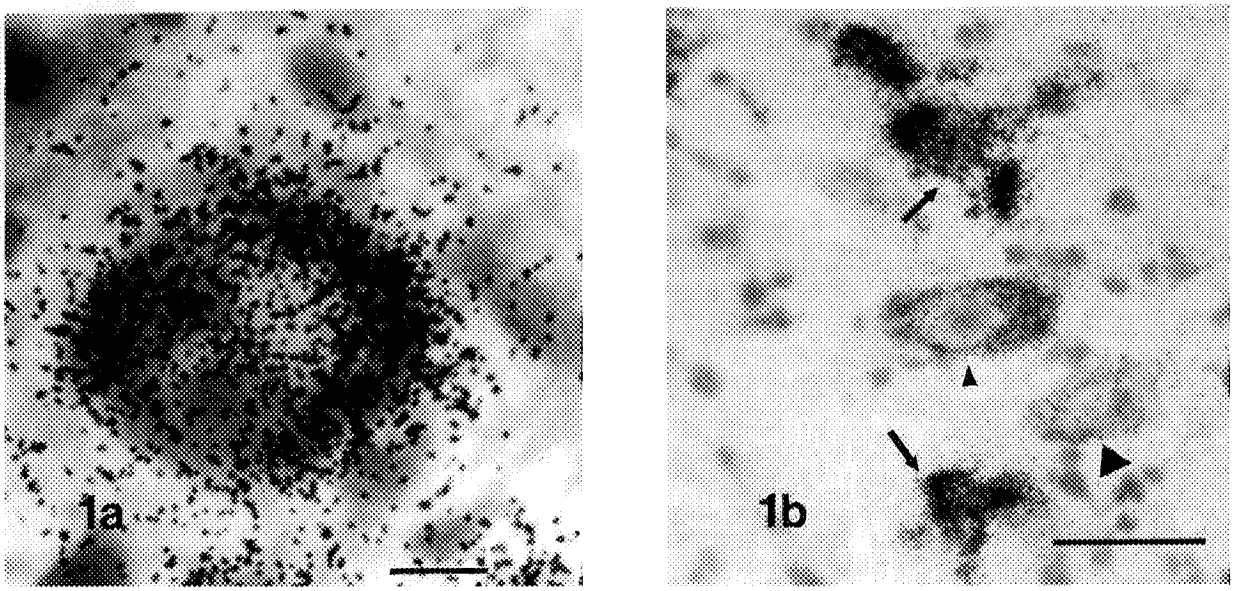

Fig 1. **a:** Hybridization histochemical preparation of a vasopressin mRNA-producing cell in the human paraventricular nucleus; silver grains predominantly overlie the neuronal cytoplasm; toluidine blue counterstain. **b:** Oxytocin mRNA-labeled neurons of the paraventricular nucleus show different labeling intensities; arrows indicate heavily labeled magnocellular neurons, small arrowhead indicates a moderately labeled neuron, and large arrowhead indicates a lightly labeled neuron. Scale bars = 10 μm in a, 50 μm in b.

aging and Alzheimer's disease (Fliers et al., '85; Swaab et al., '85; Saper and German, '87; Vogels et al., '90).

All vertebrates studied to date possess the equivalent of vasopressin- and oxytocin-like hypothalamo-neurohypophyseal systems originating in cells concentrated in the PVN and SON (Caffé et al., '89). Both peptides are found in neurons outside the hypothalamic magnocellular system (Fliers et al., '86; Ulfig et al., '90). However, despite a large body of data in a variety of species, only a few reports have been devoted to the localization of vasopressin and oxytocin in the human hypothalamus (Dierickx and Vandesande, '77, '79; Stopa et al., '84; Fliers et al., '86; Rivkees et al., '88; Ulfig et al., '90, Mengod et al., '92).

Recently, synthetic oligonucleotide probes have been used to detect messenger ribonucleic acids (mRNA) coding for specific peptides or proteins within the cytoplasm of cells, providing us with a sensitive and reliable method for visualizing neuropeptide-producing neurons in the brain (Kawata et al., '88; Young, '90). This in situ hybridization histochemical technique has been shown to be applicable to postmortem human tissue (Rance and Young, '91; Rivkees et al., '88; Walker et al., '91; Mengod et al., '92). However, there is currently little information on the anatomical distribution of mRNA for vasopressin or oxytocin in the human brain. We employed hybridization histochemistry to localize mRNA encoding vasopressin and oxytocin within neurons of the human hypothalamus and adjacent structures. This study is the first in a series of reports on the anatomical localization of transcripts for transmitter-specific markers in the human hypothalamus. Because of

Abbreviations

A	amygdala
AC	anterior commissure
AMN	accessory magnocellular nucleus
BNST	bed nucleus of the stria terminalis
dATP	deoxyadenosine triphosphate
DBB	diagonal band of Broca
DBBHL	diagonal band of Broca horizontal limb
DMN	dorsomedial nucleus
F	fornix
GPe	globus pallidus pars externa
GPi	globus pallidus pars interna
IC	internal capsule
IN	intermediate nucleus
INF	infundibular nucleus
IS	infundibular stalk
LH	lateral hypothalamus
LT	lateral tuberal nucleus
LV	lateral ventricle
MB	mammillary body
MML	medial medullary lamina
MPOA	medial preoptic area
mRNA	messenger ribonucleic acid
MT	mammillothalamic tract
NBM	nucleus basalis of Meynert
OC	optic chiasm
OT	optic tract
OXT	oxytocin
P	putamen
PHA	posterior hypothalamic area
POG	parolfactory gyrus
POR	preoptic recess
PVN	paraventricular nucleus
S	septum
SCN	suprachiasmatic nucleus
SON	supraoptic nucleus
SONdl	supraoptic nucleus, dorsolateral part
SONvm	supraoptic nucleus, ventromedial part
ST	stria terminalis
TM	tuberomammillary nucleus
VMN	ventromedial nucleus
VP	vasopressin
ZI	zona incerta
3V	third ventricle

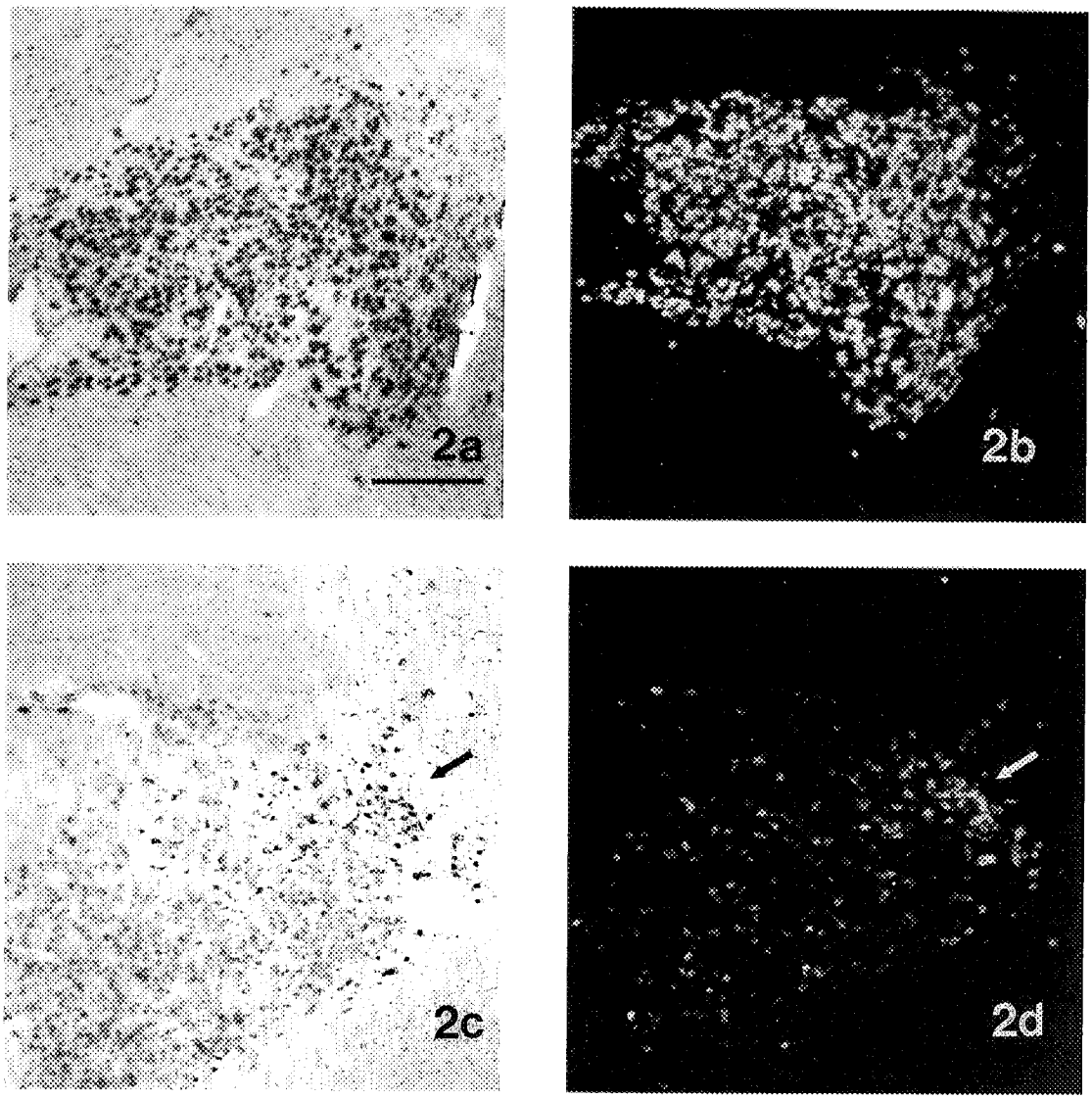

Fig. 2. Vasopressin (a,b) and oxytocin (c,d) neurons in the SONdl. **a:** Brightfield photomicrograph of vasopressin magnocellular neurons in the SONdl. Medium-sized and large, robustly labeled cells occupy the core of the nucleus. **b:** Darkfield photomicrograph of the vasopressin neurons shown in a. **c:** Brightfield photomicrograph of oxytocin magnocellular neurons in dorsolateral supraoptic nucleus. Arrow indicates intensely labeled oxytocin neurons that cluster at the medial periphery of nucleus. **d:** Darkfield photomicrograph of the oxytocin neurons shown in c. Scale bar = 300 μm for a–d.

the wealth of information on hypothalamic structure and physiology in experimental animals, the comparative analysis of neuroactive substances will provide useful information concerning the functional organization of the human hypothalamus.

MATERIALS AND METHODS

Tissue blocks were taken at autopsy from five men, 16–70 years old, with a mean postmortem interval of 9 hours. All subjects had died acutely, and complete autopsies did not disclose disorders that would be expected to compromise our analyses (Table 1). Autopsy specimens were collected in accordance with the guidelines set forth in Federal Register 46, and with the institutional guidelines of the Johns Hopkins University School of Medicine and the University of Arizona College of Medicine. Each tissue sample contained the entire hypothalamus and all or part of the following structures: parolfactory/paraterminal cortex, septum, substantia innominata, dorsal amygdala, caudate nucleus, putamen, globus pallidus, and thalamus. Blocks of fresh tissue were placed on foil-coated glass slides, frozen at −30°C in isopentane, and stored at −80°C.

Hybridization histochemistry

Sections (20 μm) were cut coronally or sagittally on a cryostat-microtome at −20°C onto gelatin-coated slides. Every twentieth section was processed for hybridization histochemistry with a specific probe, as described previously (Young, '89; Walker et al., '91). Briefly, sections were brought to room temperature, postfixed in 4% formaldehyde in phosphate-buffered saline for 5 minutes, treated

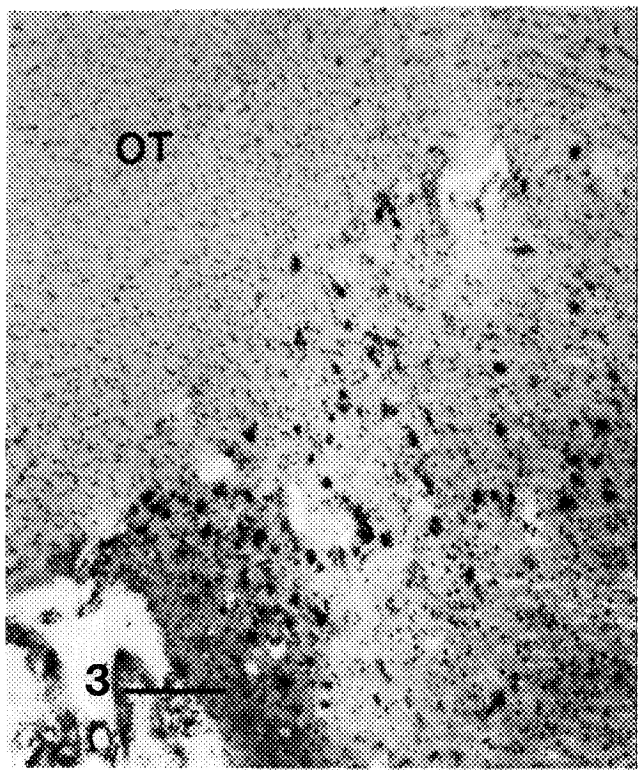

Fig. 3. Vasopressin mRNA-producing neurons in the SONvm. Cells in this subdivision of the supraoptic nucleus are sparser than in the dorsolateral part. Scale bar = 300 μm.

with 0.25% acetic anhydride for 10 minutes, and delipidated in a graded series of ethanols and chloroform. After drying, sections were incubated for 20 hours at 37°C in a buffer consisting of 600 mM NaCl, 80 mM Tris-HCl (pH 7.5), 4 mM ethylenediaminetetraacetic acid, 0.1% sodium pyrophosphate, 0.2% sodium dodecyl sulfate, 50% dextran sulfate, 0.2 mg/ml heparin sulfate, 100 mM dithiothreitol, and ~10^6 dpm of [^{35}S]-labeled probe per 50 μl (see below). Sections were then washed in a solution of 0.3 M NaCl-30 mM sodium citrate buffer, and 50% formamide at 40°C. After drying, sections were dipped in Kodak NTB-3 nuclear emulsion and exposed at 4°C for 2 or 60 days for vasopressin and 4 or 30 days for oxytocin. Sections were counterstained with toluidine blue and coverslipped.

Oligonucleotide probes were directed toward bases 1024–1068 and 2209–2256 of the human oxytocin and vasopressin preprohormone sequences, respectively (Sausville et al., '85). The vasopressin probe used in our experiments shares no similarity with oxytocin cDNA, whereas the oxytocin probe has only 62% sequence identity with vasopressin cDNA. The calculated melting temperatures under the hybridization conditions indicated above were 73°C and 75°C for the vasopressin and oxytocin probes, respectively. Oligonucleotide probes were labeled on the 3′ end using terminal deoxynucleotidyl transferase (Boerhinger-Mannheim, Indianapolis, Indiana) and [^{35}S] deoxyadenosine triphosphate (dATP) (> 1,000 Ci/mmol; New England Nuclear, Boston, MA). The specificity of hybridization was determined by Northern blot analysis of total human hypothalamic mRNA as previously described (Rance and Young, '91). Probes were labeled using [^{32}P] dATP (3,000 Ci/mmol, New England Nuclear) and terminal deoxynucleotidyl transferase. We detected RNA species of about 900 and 700 base pairs with the vasopressin and oxytocin probes, respectively, similar to values reported previously for the human (Mohr et al., '85).

TABLE 1. Perimortem Histories of Human Subjects

Human case #	Sex	Age (years)	Cause and time of death	Postmortem interval (hours)
426	Male	61	Acute myocardial infarction; 10:40 p.m.	13
810	Male	16	Lacerated lung; 8:20 p.m.	12
814	Male	52	Asphyxiation; 7:45 a.m.	4
871	Male	23	Gunshot wound; 3:00 a.m.	7
914	Male	70	Acute duodenal hemorrhage; 5:55 a.m.	9.5

Analysis of tissue

Sections were mapped with a computer-assisted microscopic mapping system (Walker et al., '90) with the aid of a 20× objective (Zeiss Axiophot). Neuronal perikarya were considered to contain vasopressin or oxytocin mRNA if the density of superimposed silver grains exceeded three times that of the surrounding neuropil. In some cases, labeling was so intense that it obscured cellular detail (Fig. 1). Lightly labeled neurons were mapped only if the grains within neuronal somata exceeded three times background and the nucleus of each cell was present (Fig. 1b). The presence of the nucleus allowed us to distinguish between actual lightly labeled neurons and peripherally sectioned, heavily labeled neurons. Cell size (maximum length and width) in the hypothalamus was determined with a calibrated eyepiece micrometer at a total magnification of 400×. All measurements were made in coronal sections, except for suprachiasmatic neurons, which were measured in sagittal sections. The identification of structures was aided by several sources (Le Gros Clark, '36; Le Gros Clark et al., '38; Nauta and Haymaker, '69; Braak and Braak, '87; Gai et al., '90; Saper, '90).

RESULTS

Vasopressin

Most neurons containing vasopressin mRNA are located in the principal magnocellular hypothalamic nuclei, i.e., SON and PVN (Figs. 4a–c, 6a–c). A smaller population of vasopressin neurons occupies the accessory magnocellular nucleus (AMN), a group of cellular islands in the region bridging the SON and PVN (Saper, '90). Generally, magnocellular neurons containing vasopressin transcripts tend to locate more centrally in these nuclei than do oxytocin neurons. Outside the magnocellular nuclei, the suprachiasmatic nucleus (SCN) contains small, lightly labeled cells, and vasopressin neurons are scattered throughout the caudolateral hypothalamus; very rarely, positive neurons are found within the boundaries of other nuclei. Each population is discussed in detail below.

Vasopressin mRNA in the SON

The human SON consists of two main subpopulations of neurons, that is, a relatively dense cluster of cells dorsal to the optic chiasm (dorsolateral SON [SONdl]) (Figs. 2a,b, 4a–c, 6c), and a more diffuse cluster ventromedial to the chiasm (ventromedial SON [SONvm]) (Figs. 3, 4a–c, 6c).

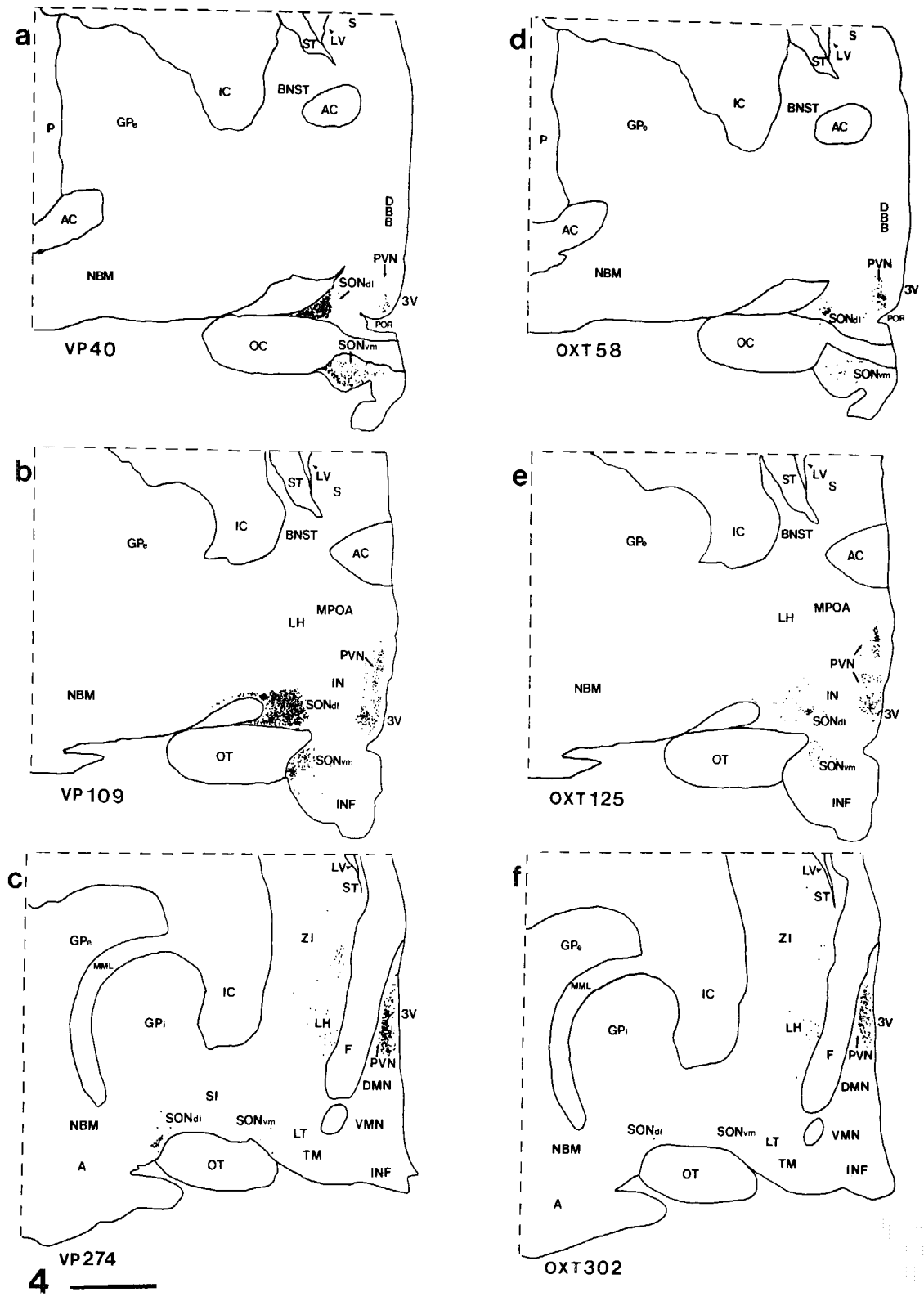

Fig. 4. Computer-assisted diagrams of the distribution of vasopressin (**a–c**) and oxytocin (**d–f**) cells in coronal sections of human hypothalamus and surrounding structures (case 814). The most anterior sections are a and d; the most posterior sections are c and f. Each dot represents a single neuron. Numbers at the lower left correspond to the relative location of the sections from anterior to posterior. Each section is 20 μm thick; hence, the distance between VP40 and VP274 is ~4.7 mm. Scale bar = 5 mm.

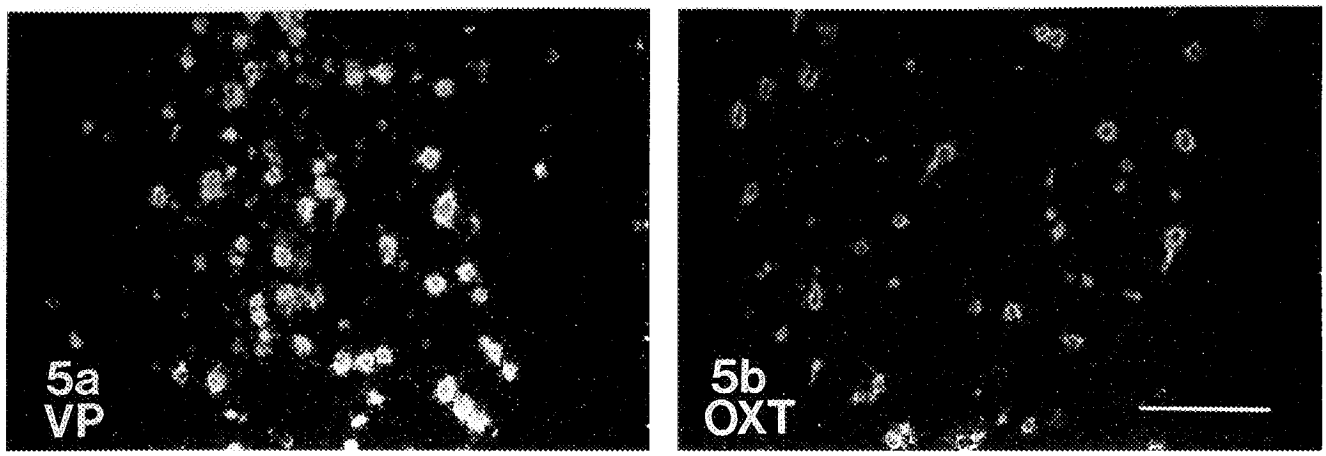

Fig. 5. Darkfield photomicrographs of nearby sections with vasopressin (**a**) and oxytocin (**b**) mRNA-containing neurons in the caudal part of the PVN. Note that the vasopressin neurons are more abundant in the central part of the PVN, whereas neurons containing oxytocin transcripts tend to have a more peripheral location. Scale bar = 200 μm.

Proceeding caudally from the anterior SON, both groups maintain their proximity to the optic tract. As the optic tract merges with the basal forebrain, the dorsal cells assume a more lateral position, and the ventral cells become clustered near the medial aspect of the optic tract (Fig. 4a-c). Most neurons in both subpopulations are robustly labeled for vasopressin mRNA. The dorsolateral group contains more densely packed large and medium-sized cells, with a preponderance of typical round or ovoid magnocellular somata, whereas the ventromedial group is characterized by somewhat sparser, morphologically more heterogeneous neurons. The mean size of vasopressin cells in SONdl was 38 μm long × 28 μm wide (n = 41 cells); in SONvm, 34 μm long × 23 μm wide (n = 54).

Vasopressin mRNA in the PVN

The PVN, adjoining the lateral wall of the third ventricle, has a high concentration of neurons with vasopressin transcripts (Fig. 5a). In sagittal sections, these cells can be seen to form a continuous band running from a ventral position in the rostral hypothalamus to a more dorsal position in the caudal hypothalamus (Figs. 6a, 7a). The large, rostroventral-most cells of the PVN (Fig. 4a) lie just medial to the smaller neurons of the SCN. Proceeding caudally, the PVN population shifts dorsally and increases in size (Figs. 4b,c, 6b). Viewed in coronal sections, vasopressin somata of the PVN are large (37 μm × 28 μm; n = 72), ovoid or round cells. These neurons are cytologically similar to magnocellular vasopressin neurons in the SON and AMN.

Vasopressin mRNA in the AMN

Cells of the AMN do not differ morphologically from the typical vasopressin neurons of the SON and PVN (Fig. 8a). Many of these neurons surround blood vessels that run between the dorsomedial SON and the ventrolateral PVN.

Vasopressin mRNA in the SCN

SCN neurons containing vasopressin mRNA were seen only in two cases (810 and 914). Viewed in sagittal sections, vasopressin neurons of the SCN had small (15 μm × 10 μm; n = 25), round or ovoid somata and were only weakly labeled (Fig. 9). There was no apparent relationship between time of death (Table 1) and the presence or absence of labeling in the SCN.

Vasopressin mRNA in the lateral hypothalamus

Vasopressin neurons in the lateral hypothalamus lie posteriorly, lateral to the column of the fornix (Fig. 4c). In the dorsoventral axis, scattered vasopressin cells extend in a narrow band from just beneath the bed nucleus of the stria terminalis to the level of the tuberomammillary nucleus and the area dorsal to the optic tract. Sparse neurons occur further laterally in the hypothalamus, just medial to the internal capsule and globus pallidus, and a few appear to stray into the region of the zona incerta (Fig. 4c). Most of the labeled lateral hypothalamic neurons are as intensely positive as vasopressin neurons of the PVN; however, morphologically, cells in the lateral hypothalamus are usually more oblong and somewhat smaller (37 μm × 20 μm; n = 41) (Fig. 10) than PVN vasopressin cells.

Extrahypothalamic vasopressin mRNA

As mentioned above, occasional vasopressin neurons of the hypothalamus appear to infiltrate adjacent structures, including the zona incerta and bed nucleus of the stria terminalis (BNST). We also detected rare, lightly labeled vasopressin cells in the nucleus of the diagonal band of Broca and in the anterior nucleus basalis of Meynert (NBM). In these latter two nuclei, vasopressin cells were not seen in all cases, and exposure time of the emulsion in some instances could have been too brief to detect lightly labeled cells. Positively labeled vasopressin neurons were evident only in sections that were exposed with emulsion for two months (Fig. 11). Even in this case, the incidence of labeled cells in the NBM and nucleus of the diagonal band was extremely low.

Oxytocin

The distribution of neurons containing oxytocin mRNA in the hypothalamus is somewhat more restricted than that

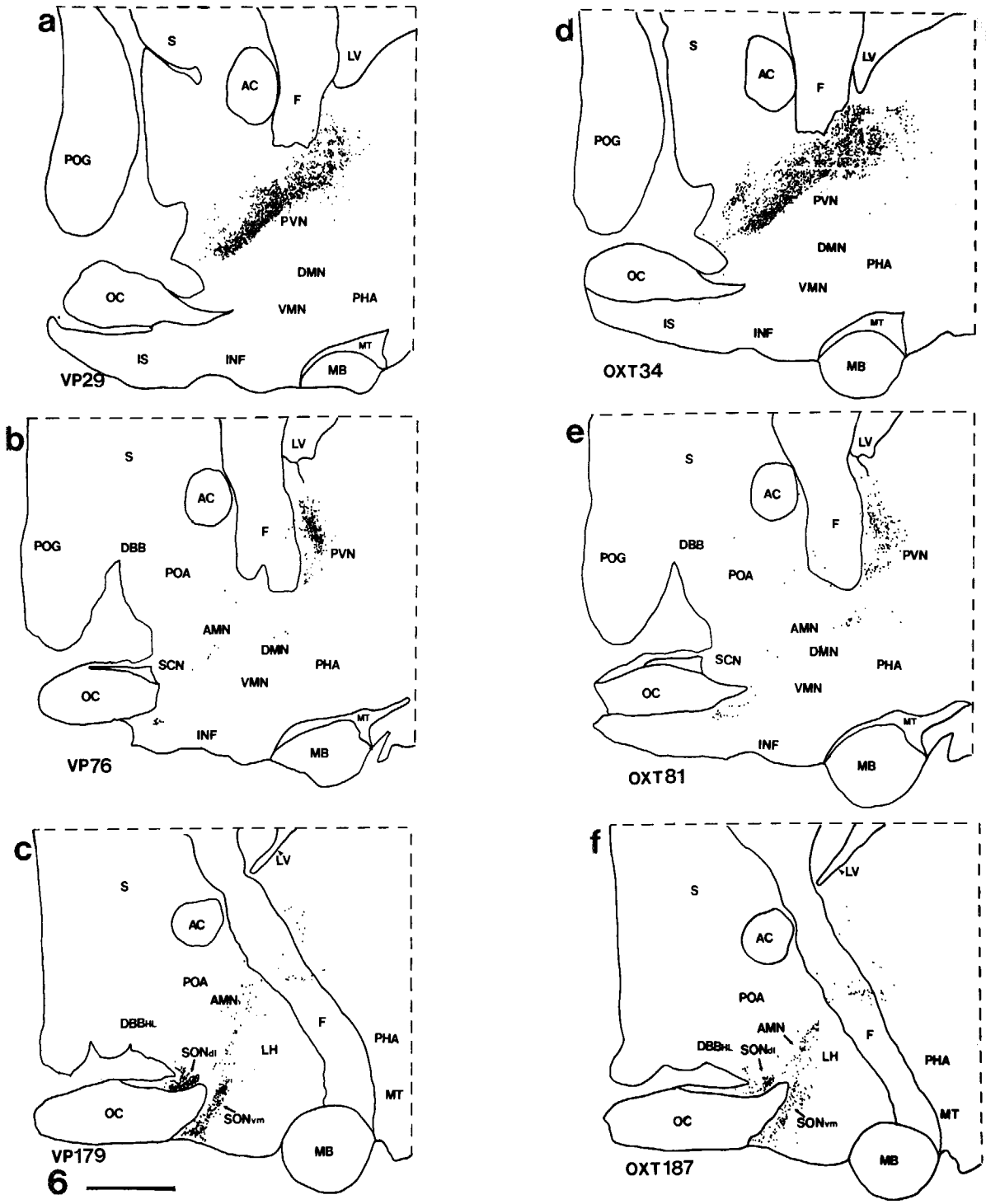

Fig. 6. Computer-assisted diagrams of the distribution of vasopressin (**a–c**; case 814) and oxytocin (**d–f**; case 871) producing neurons in sagittal sections of the human hypothalamus. The most medial sections are a and d; the most lateral sections are c and f. Each dot represents a single neuron. Sagittal sections are numbered serially from medial to lateral; each section is 20 μm thick. Scale bar = 5 mm.

of vasopressin neurons. There are also some subtle differences in the distribution, density, and size of oxytocin and vasopressin neurons within the hypothalamic magnocellular complex. Oxytocin neurons are located in the SON, PVN, and AMN (Figs. 4d–f, 6d–f) but tend to predominate in peripheral parts of these nuclei. As with vasopressin, lateral hypothalamic oxytocin neurons occasionally appear to impinge on nearby nuclei, but not in significant numbers.

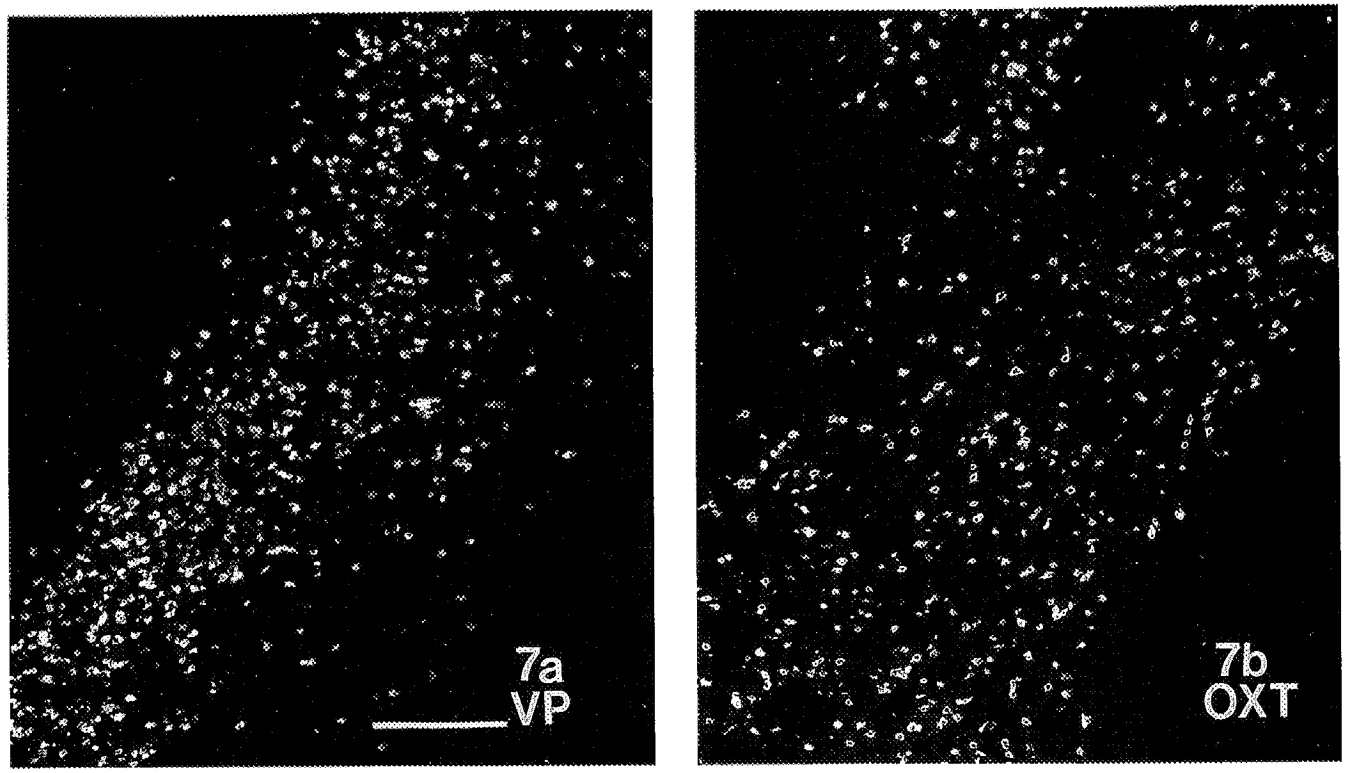

Fig. 7. Darkfield photomicrographs of nearby sagittal sections showing the gene expression of vasopressin (**a**) and oxytocin (**b**) in PVN; rostral is to the left, and ventral is down. Scale bar = 740 μm.

Oxytocin mRNA in the SON

Oxytocin mRNA is found in neurons throughout the SON and can be divided into two subpopulations based on size and hybridization intensity. One subpopulation consists of typical large, round or ovoid neurons of the SONdl and, to a lesser extent, the SONvm, most of which are relatively lightly labeled. A second subpopulation is formed of intensely oxytocin mRNA-positive neurons along the medial border of the SONdl (Fig. 2c,d); scattered, heavily labeled cells also are found among large cells of the SONdl proper and in the SONvm. Intensely labeled neurons of the SONdl are, on average, slightly smaller (26 μm × 18 μm; n = 57) than more lightly labeled oxytocin cells (35 μm × 25 μm; n = 38) of the SON. Although the heavily labeled subpopulation of SON cells was comparable in all cases studied, the percentage of lightly reactive neurons within the SONdl varied among cases. Cells containing oxytocin mRNA were less numerous than those containing vasopressin mRNA in the SON.

Oxytocin in the PVN

Neurons containing oxytocin mRNA are found throughout the PVN and are approximately equal in number to vasopressin neurons. Despite considerable intermingling, oxytocin and vasopressin cells tend to segregate to some degree (Fig. 5). On the whole, oxytocin neurons in the PVN are more widely dispersed than vasopressin cells, as can best be seen in sagittal sections (Figs. 6d,e, 7). On average, oxytocin neurons are smaller (28 μm × 19 μm; n = 74) than vasopressin neurons in the PVN.

Oxytocin in the AMN

As with vasopressin cells, scattered clusters of oxytocin neurons populate the AMN, and labeled cells frequently surround blood vessels. Furthermore, oxytocin cells and vasopressin cells appear to segregate within the AMN islands (Fig. 8b).

Oxytocin mRNA in the lateral hypothalamus

Oxytocin neurons in the lateral hypothalamus are similar in morphology and organization to vasopressin neurons, except that oxytocin neurons are somewhat fewer in number (Fig. 4f).

DISCUSSION

Using sensitive and specific oligonucleotide probes, we have examined the distribution of neurons that express vasopressin and oxytocin in the human hypothalamus. Our findings largely confirm previous immunocytochemical studies on the hypothalamic localization of these neuropeptides in humans and nonhuman primates (Dierickx and Vandesande, '77, '79; Stopa et al., '84; Swaab et al., '85; Caffé et al., '89; Saper, '90). In the magnocellular hypothalamic complex, which includes SON, PVN, and AMN, numerous neurons contain vasopressin or oxytocin mRNA, and these cell types tend to segregate to some degree (Figs 2, 5, 7, 8). Studies in experimental animals have demonstrated colocalization of vasopressin and oxytocin in hypothalamic neurons as a result of salt-loading or lactation (Kiyama and Emson, '90; Mezey and Kiss, '91). Although we were unable

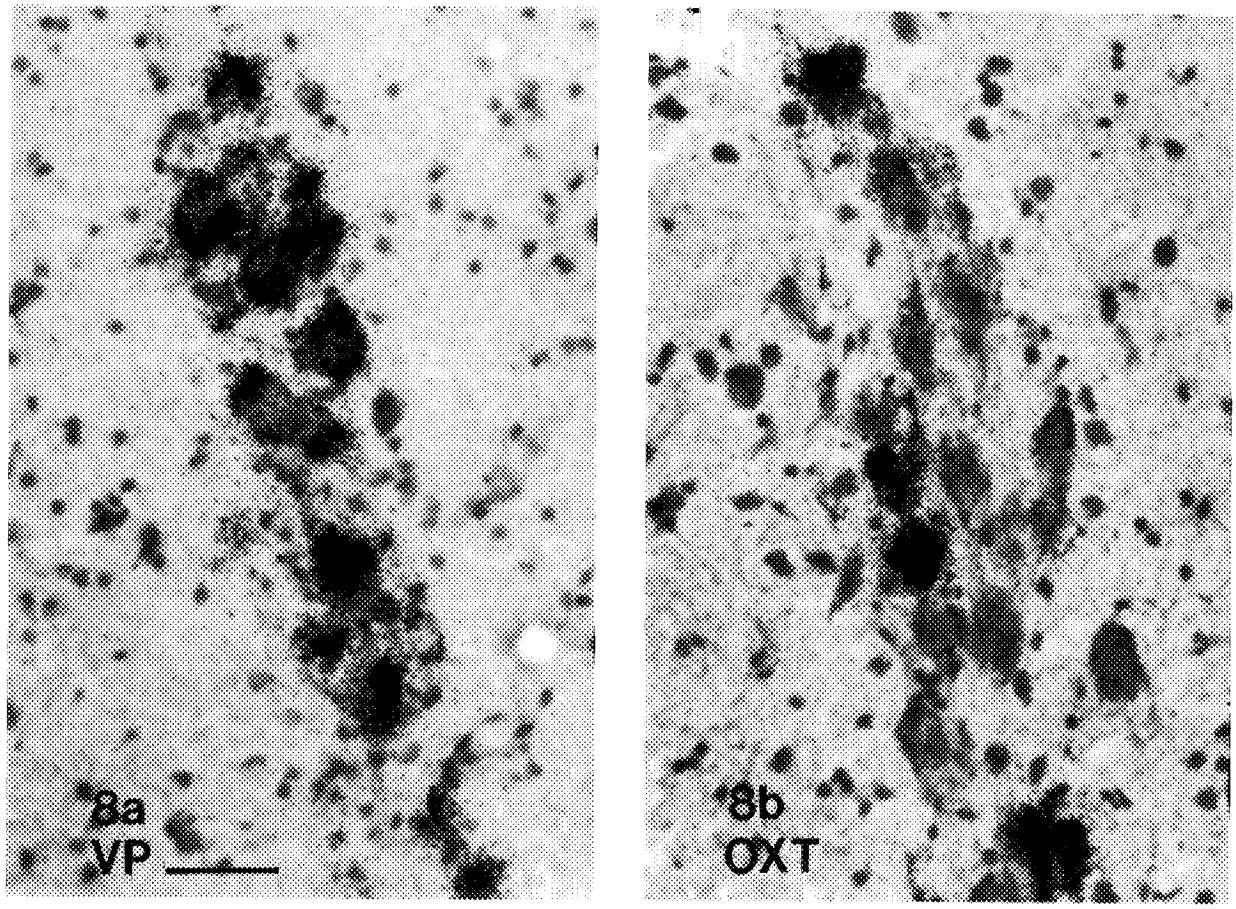

Fig. 8. Vasopressin (**a**) and oxytocin (**b**) neurons in the accessory magnocellular nucleus (case 426). Accessory magnocellular neurons often surround blood vessels in the area lying between the SON and PVN. Scale bar = 40 μm.

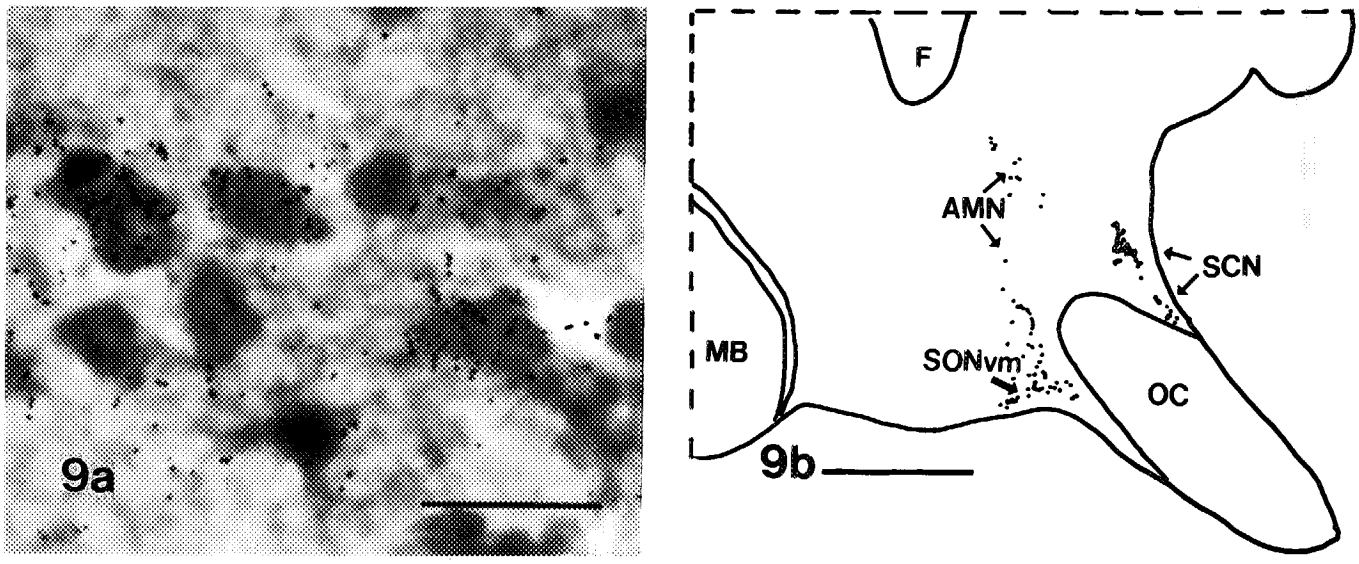

Fig. 9. **a:** Small, lightly labeled vasopressin neurons in the suprachiasmatic nucleus (case 810). **b:** Schematic, parasagittal diagram of the hypothalamus in case 810 showing the location of the suprachiasmatic nucleus (SCN). Scale bars = 25 μm in a, 5 mm in b.

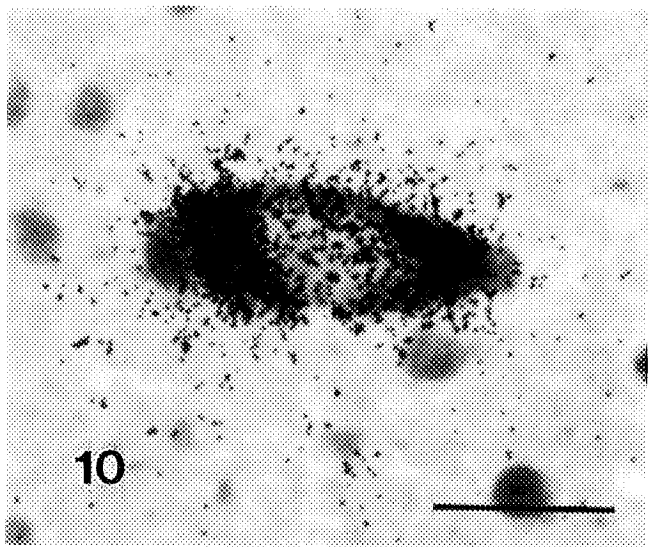

Fig. 10. Lateral hypothalamic neuron containing vasopressin transcripts. Scale bar = 25 μm.

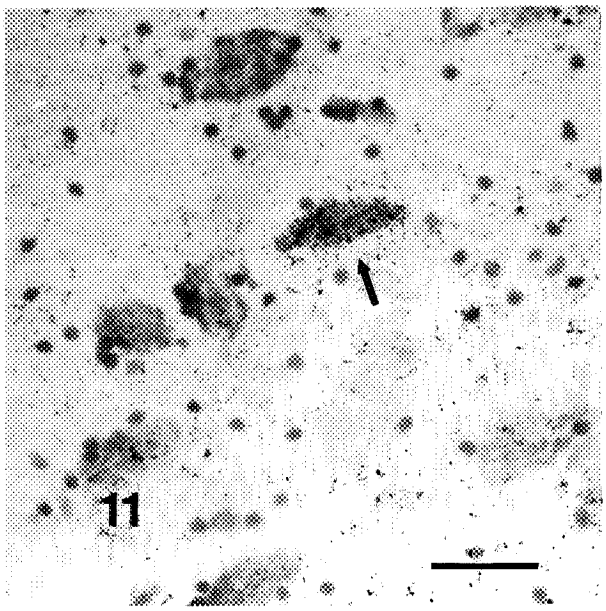

Fig. 11. Vasopressin mRNA-positive neuron (arrow) in the NBM; neurons containing vasopressin transcripts were very rare in the nucleus basalis and in the nucleus of the diagonal band of Broca. Scale bar = 35 μm.

to address the issue of whether oxytocin and vasopressin genes are expressed within the same cells, the inclination of oxytocin and vasopressin neurons to segregate within magnocellular nuclei, and to differ in size, suggests that the peptides may not colocalize to a large extent in humans.

It is also important to determine whether oxytocin and vasopressin colocalize with other neuroactive substances in the human hypothalamic magnocellular nuclei. In rodents, immunocytochemical studies have yielded evidence that vasopressin-containing neurons can express tyrosine hydroxylase, galanin, dynorphin, leucine enkephalin, or peptide histidine isoleucine, whereas oxytocin-containing neurons also can express cholecystokinin or corticotropin-releasing factor (Brownstein and Mezey, '86; Meister et al., '90). Recently, with the development of nonradioactive in situ hybridization histochemical methods, it has become possible to study the coexpression of mRNAs using digoxigenin- and [^{35}S]-labeled probes (Young and Hsu, '91). This approach could be used to confirm whether transcripts that encode different peptides colocalize within individual neurons of the human brain.

The magnocellular vasopressin- and oxytocin-containing nucleus shows marked sexual dimorphism in pigs (van Eerdenburg and Swaab, '91). Furthermore, the expression of oxytocin mRNA in SON and anterior commissural nucleus is increased by estrogen treatment in ovariectomized rats (Chung et al., '91). We could not address the issue of sex differences in our subjects, who were all males, but the influence of gender on the expression of hypothalamic peptides in humans remains an important issue for future research.

With some exceptions, notably the SON and PVN, most cell groups of the adult human hypothalamus are rather poorly delineated in Nissl-stained sections (Saper, '90). One nucleus that is not clearly defined is the SCN, a structure important in regulating biological rhythms (Moore, '91). Vasopressin cells have been reported in the human SCN by means of immunocytochemistry (Dierickx and Vandesande, '77; Stopa et al., '84; Swaab et al., '85) and in situ hybridization (Rivkees et al., '88). We observed vasopressin gene expression in neurons of the SCN only in two cases. The cells were small and lightly labeled (Fig. 9), and they were clearly distinguishable from the large, heavily labeled cells of the rostroventral PVN. The inconsistency of gene expression in the SCN of our cases remains mysterious, but one potential explanation is the circadian rhythmicity of the SCN, which has been well documented in experimental animals (Carter and Murphy, '89, '91). SCN neurons display significant circadian variation in the expression of vasopressin transcripts (Uhl and Reppert, '86); vasopressin peptide levels in cerebrospinal fluid also show a rhythmic, daily pattern in cats (Reppert et al., '81). Although there was no systematic relationship between time of death and

the presence of vasopressin labeling in the five cases that we studied, the possibility that premortem stimuli may influence the expression and/or degradation of mRNA in the SCN deserves further study.

We observed diffuse populations of vasopressin and oxytocin mRNA-producing neurons in the lateral hypothalamus (Fig. 4c,f) that have not been described elsewhere. Because these scattered cells appear to disregard conventional anatomical boundaries, their nuclear affiliations are uncertain. The function and connectivity of these cells also remain to be determined.

Extrahypothalamic vasopressin-immunoreactive neurons have been reported in the BNST (Fliers et al., '86) and in some parts of the NBM complex (Ulfig et al., '90) in humans, and also in monkeys (Caffé et al., '89). Only a few positive neurons of the dorsal-most part of the lateral hypothalamus extended into the caudal BNST in our material; furthermore, the overwhelming majority of cells in the NBM complex are devoid of vasopressin transcripts. Thus, although only portions of these structures were present in our sections, we were unable to confirm the existence of significant subpopulations of vasopressin neurons in either the NBM complex or the BNST of humans.

In summary, our hybridization histochemical data supplement previous immunocytochemical data on the distribution of oxytocin and vasopressin in the human hypothalamus. The results suggest that this sensitive and specific method is a useful means of revealing the neurochemical identities of neurons. In ongoing studies, we are analyzing the neuroanatomy of gene expression for other neuroactive substances in the hypothalamus to establish the comparability of these systems in humans and other mammals. This information is essential to our eventual understanding of how the human hypothalamus carries out its myriad functions.

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